THE BIOLOGY AND CULTIVATION OF OYSTERS IN AUSTRALIA.

IV. OYSTER CATALASE.*

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(Two Text-figures.)

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Introduction.

Previous work on oyster catalase consists only of that of Hopkins (1930, 1934), in which an attempt was made to estimate the catalase content of the adductor muscles of various molluscs and to relate the values so obtained to age and O_2 consumption. However, for the estimations, he used a manometric method, the results from which are apt to be rather low. Moreover, there was no attempt to adjust the pH of the reaction mixture to any definite value, and the substrate concentration was about 0·1 M instead of in the region of 0·01 to 0·02 M. These points are particularly important since it has been shown that the course of the reaction is affected by the substrate and buffer concentrations, and also, that the enzyme is destroyed by relatively high substrate concentrations, especially in the absence of buffers (Morgulis, 1931).

It seemed desirable also, in connection with the programme already outlined (Kesteven and Humphrey, 1941) to investigate some of the properties of the catalase in Australian oysters.

Methods.

The method used for the estimation of catalase was based on that of Okey (1922).

In a 150 ml. Erlenmeyer flask were placed 10 ml. of buffer solution, 10 ml. of 0·15 M H_2O_2 and a volume of distilled H_2O which, when the enzyme solution was finally added, would make the total volume 100 ml. The flask was then brought to the required temperature by leaving in a thermostat for 15 minutes, the enzyme solution added and the contents mixed. Aliquots of 10 ml. were withdrawn as required, run into 30 ml. of 5 N H_2SO_4 and titrated with $KMnO_4$ (0·05–0·10 N). For the preparation of the enzyme solutions, several methods were used:

1. The weighed, fresh tissue was ground (plus sand, if necessary) with five times its weight of distilled H_2O and let stand at room temperature overnight. The suspension was then filtered through a No. 1 Whatman paper and washed with H_2O to bring the strength of the crude extract to 10%.

2. An attempt was made to prepare a concentrated solution by the method of Keilin and Hartree (1936), as modified by Bertho and Grassmann (1938), but the final solution after dialysis showed no activity.

3. An attempt was also made to prepare crystalline catalase by the dioxane fractionation according to Sumner and Dounce (1939), but without success.

4. Some fresh oysters were taken and opened; 230 gm. of the meats were minced and extracted overnight in the refrigerator with 1 litre of distilled H_2O. The mixture was then squeezed through muslin and the thick fluid centrifuged. The supernatant was then filtered through a No. 1 Whatman paper. The filtrate was treated with varying amounts of saturated $(NH_4)_2SO_4$ solution and kept in the refrigerator overnight to discover the concentration of $(NH_4)_2SO_4$ appropriate for fractionating the

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extract. The total volume was 50 ml. in each case and $CHCl_3$ was added as a preservative except in No. 8, which was kept as a control on the effect of $CHCl_3$.

The precipitates were filtered off next day and the activities of the filtrates were determined at 25°C. in the presence of citrate buffer of pH 6·7 (Gortner, 1938). For the addition of the enzyme, an amount was taken from each filtrate to correspond with 0·5 ml. of the original extract. The results were as follows:

TABLE 1.

Number.	$(NH_4)_2SO_4$ Saturation.	$CHCl_3$.	Ml. Filtrate used for Estimation.	Mg. H_2O_2 decomposed at	
				6 min.	15 min.
1	1·00	+	0·50	0·0	0·0
2	0·75	+	2·00	15·5	23·8
3	0·67	+	1·50	7·7	13·1
4	0·50	+	1·00	37·4	45·9
5	0·33	+	0·75	40·5	47·5
6	0·25	+	0·67	41·2	47·3
7	0·00	+	0·50	40·0	47·2
8	0·00	—	0·50	39·9	46·6

It can be seen, therefore, that between 0·50 and 0·67 saturation with $(NH_4)_2SO_4$ the critical precipitation of catalase occurs.

A mixture of 50 ml. of the original extract and 50 ml. of saturated $(NH_4)_2SO_4$ was kept in the refrigerator overnight and then filtered; a half volume of saturated $(NH_4)_2SO_4$ was then added to the filtrate and the mixture left in the refrigerator overnight. It was next centrifuged for 20 minutes at about 2,500 r.p.m. and washed with 5 ml. of 0·67 saturated $(NH_4)_2SO_4$ and centrifuged as before. The supernatant was then decanted and the residue dissolved in 12·5 ml. of citrate buffer (pH = 6·7). The clear solution was filtered and the filtrate kept as the "purified enzyme solution"; it gave no colour with iodine solution, thus indicating the absence of glycogen.

For controls 0·1 ml. of 0·1 M NaCN was added to the reaction mixture; there was never any peroxide decomposition in such mixtures.

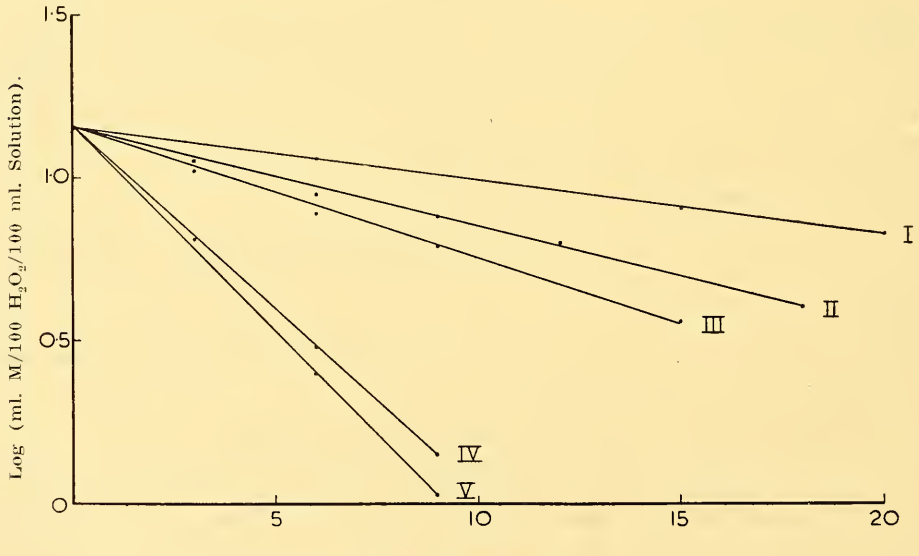

Fig. 1.—Progress curves for different organs using 1 ml. of 10% extract at 0°C. in phosphate buffer (pH = 7·0). I, Adductors. II, Mantle and gills. III, Digestive diverticula. IV, Gonads. For comparison, V, with 0·2 ml. of purified enzyme solution at 0°C. in citrate buffer (pH = 6·7), is included.

Results.

In Fig. 1 are given the results obtained with crude extracts from different parts of the one oyster. The straight lines indicate a first-order reaction. A number of oysters have been thus examined; in all cases the gonad extract is the most powerful, then the digestive diverticula, but sometimes the adductor extract is stronger than that of the mantle and gills. For comparison, there is included in Fig. 1 a curve for the purified enzyme solution.

Fig. 2 shows the curves obtained with different quantities of the one enzyme solution; these also indicate reactions of the first order.

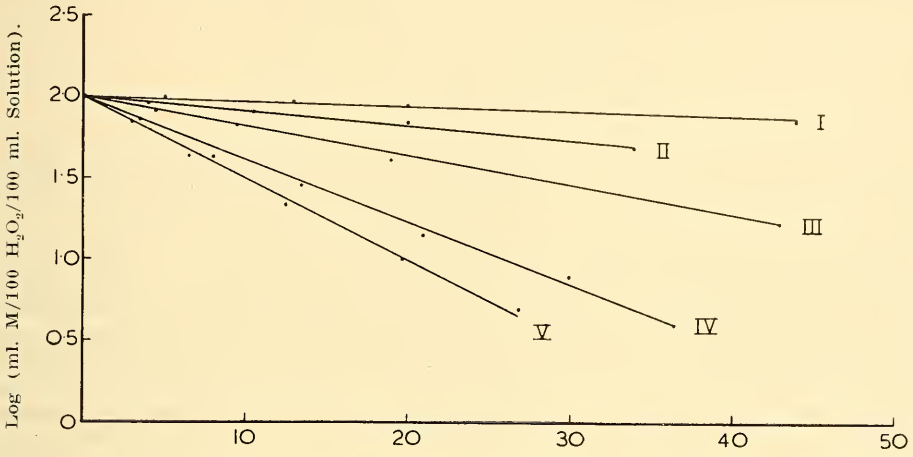

Fig. 2.—Progress curves for different quantities of extract, using 10% gonad extract at 0°C. in phosphate buffer (pH = 7·0). I, 0·1 ml. II, 0·2 ml. III, 0·4 ml. IV, 0·8 ml. V, 1·0 ml.

Table 2 shows the results of an experiment designed to determine the pH optimum of the purified enzyme solution.

Table 2.

0·2 ml. of Purified Enzyme Solution in Citrate Buffer at 25° C.

pH.	Mg. H_2O_2 decomposed at	
	6 min.	15 min.
1·2	0·0	0·0
2·3	0·0	0·0
3·4	0·0	0·0
4·4	4·0	6·7
5·6	15·0	24·2
6·7	15·5	25·5
9–10	11·0	19·9
12·4	4·0	4·6

The pH optimum lies, therefore, around 6·7. Another experiment was carried out in the range 5·6 to 8·0; this time with phosphate buffer (M/15 Na_2HPO_4 and M/15 KH_2PO_4).

Table 3.

0·2 ml. of Purified Enzyme Solution in Phosphate Buffer at 25° C.

pH.	Mg. H_2O_2 decomposed at	
	6 min.	15 min.
5·6	10·8	16·9
6·0	11·7	18·1
6·7	12·8	18·6
7·0	12·3	18·3
8·0	12·0	17·2

Therefore the pH optimum is located at 6·7, although the enzyme works almost as well at pH = 7·0. It is interesting to note that at a given pH the enzyme acts better in citrate than in phosphate buffer.

The effect of temperature on the reaction is indicated below.

TABLE 4.
0·2 ml. of Purified Enzyme Solution in Citrate Buffer at pH = 6·7.

Temperature.	Mg. H_2O_2 decomposed at	
	6 min.	15 min.
0° C.	12·1	23·6
15° C.	13·2	25·3
25° C.	14·5	23·7
35° C.	6·2	7·8

Only at 35°C. is there any great diminution of activity. Over a short period (6 min.) 25°C. gives the most action, but for a longer period (15 min.) 15°C. is better.

Discussion.

The only other marine invertebrate which has been at all fully investigated is the Californian mussel (Marks and Fox, 1934). Apart from the temperature optimum and the fact that these workers used crude extracts all the time, the properties of the catalase investigated by them are essentially similar to those of the catalase from *Saxostrea commercialis*. These workers also found that in the case of the mussels, the extracts prepared from the digestive diverticula were much more powerful than those prepared from the gonads; in the present investigation the reverse is true. However, the distribution of catalase in organisms such as mussels and oysters is, conceivably, related to their sexual state, and factors such as this must be taken into account before deductions can be drawn concerning the relative significance of catalase in different organs.

Okey (1922) states that $CHCl_3$ accelerated the activity of blood catalase by 10%; no such effect was found with the present preparation.

Bertho and Grassmann (1938) state that the reaction with a substrate concentration of 0·015 M is monomolecular; this was found to be the case with all concentrations of enzyme used (Fig. 2).

Summary.

A purified catalase solution has been obtained from the Australian oyster, *S. commercialis*.

The pH optimum was 6·7 at 25°C. in the presence of either citrate or phosphate buffer. The enzyme works better in citrate than in phosphate buffer over the pH range 5·6 to 6·7.

The reaction studied seems to be of the first order.

Acknowledgment.

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